CO-SELECTION OF ANTIBIOTIC RESISTANCE IN GRAM-NEGATIVE BACTERIA CAUSED BY POLLUTION LEGACY IN THE CLYDE ESTUARY

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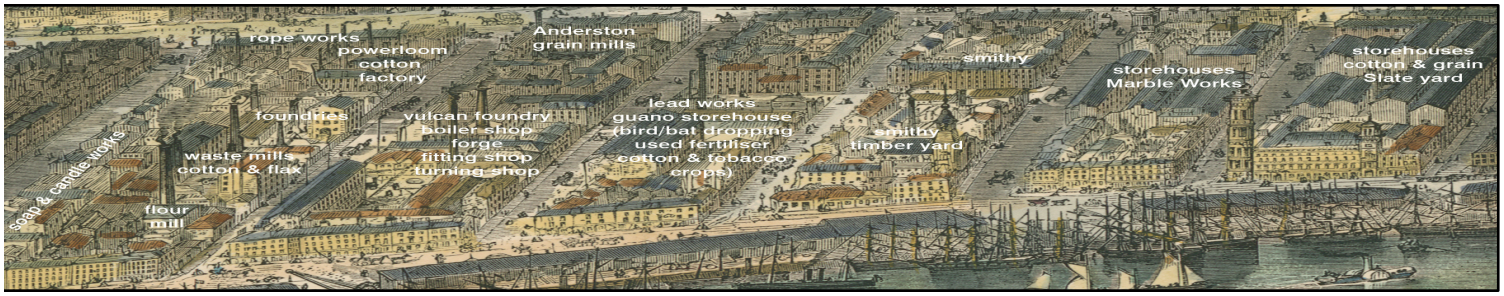
HYPOTHESIS

In the Clyde, UK co-selection antibiotic resistance occurs in microorganisms, promoted by stress from potentially toxic element (PTE) presence, and impacts bacteria that could possibly be human pathogens. Higher concentrations of PTE in the environment correlates to higher MIC to the PTE, and ultimately to higher antibiotic resistance levels.



INTRODUCTION

Recent evidence indicate that numbers of antibiotic resistant bacteria within contaminated landscapes are significantly higher than that of uncontaminated land.
The legacy of pollution and the inability to remove pollution by-products such as heavy metals can cause co-selection for antibiotic resistant genes within bacterial species.
Three sites with different pollution levels were chosen to compare the levels of antibiotic/heavy metal resistance genes:
Clydebank, Dumbarton West and Cardross.
Using sediment cores, we were able to get a historical representation of pollution and resistant bacteria levels from the past to the present

PCA ANALYSIS OF PTE VALUES IN CLYDE SITES

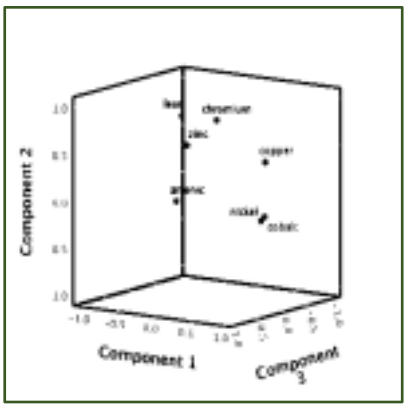


Fig. 1- PCA analysis of the PTE values in Cadross, Dumbarton West and Clydebank

In order to determine the correlation between the potentially toxic elements found within the 3 chosen sites principal component analysis was used. Where the values lie between the 3 components highlights the variation between source of pollution.

96-WELL SUSCEPTIBILITY ASSAY & qPCR

96-well assays were read spectro-photometrically to determine IC50 value. Minimum Inhibitory Values were visualized by using reazurin as an oxidizing reagent and Minimum Bactericidal Concentrations were re obtained by plating the wells which showed no colour change (and subsequently no viability of bacteria).

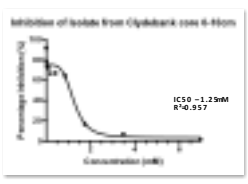


Figure 2-IC50 Curve of Clydebank Core 0-10cm- Calculating the percentage inhibition to Nickel Chloride presented by the isolate from the Clydebank Site

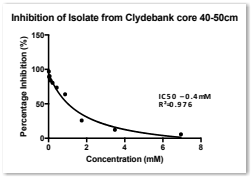


Figure 3 -IC50 Curve of Clydebank Core 40-50cm- Calculating the percentage inhibition to Nickel Chloride presented by the isolate from the Clydebank Site

qPCR

Real–time PCR was used to detect the presence of metal resistance genes from extracted DNA. This allowed comparisons to be made between metal gene abundance, the resistance genes found in high throughput gene array qPCR and the Minimum-Inhibitory Concentrations obtained.

MINIMUM INHIBITORY CONCENTRATION & qPCR RESULTS

Minimum Inhibitory Results were compared to PTE levels in the soils and their depths to identify if a correlation between the organism isolated and the resistance that organism showed to the individual PTE upon re-exposure. An example of the data has been given below.

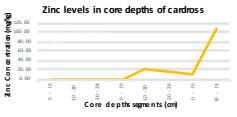


Figure 3 - Concentration of Zinc at varying core depths at Cardross, River Clyde UK

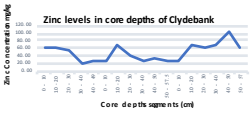


Figure 4 – Concentration of Zinc at varying core depths at Clydebank, River Clyde UK

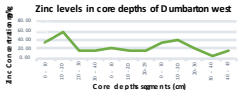


Figure 5 - Concentration of Zinc at varying core depths at Dumbarton West, River Clyde UK.

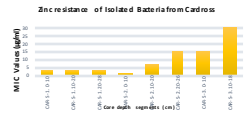


Figure 6 -Minimum Inhibitory Concentration of Cardross Isolates to Zinc Sulfate.

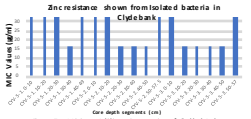


Figure 7 – Minimum Inhibitory Concentration of Clydebank Isolates to Zinc Sulfate

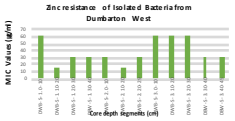


Figure 8 – Minimum Inhibitory Concentration of Dumbarton West Isolates to Zinc Sulfate

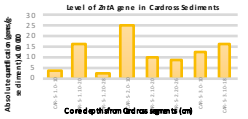


Figure 9 - The abundance of ZntA gene (copies per gram of soil DNA) quantified by qPCR) in Cardross Core Depths.

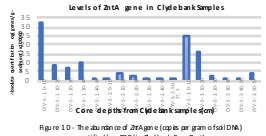


Figure 10 - The abundance of ZntA gene (copies per gram of soil DNA) quantified by qPCR) in Clydebank Core Depths.

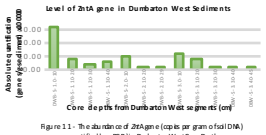


Figure 11 - The abundance of ZntA gene (copies per gram of soil DNA) quantified by qPCR) in Dumbarton West Core Depths.

Higher MICs in isolated organisms from sediments with higher levels of zinc in the sediments. These results also provides some correlation between the absolute levels of the zinc resistant gene ZntA found in the sites via quantitative PCR for the gene.

HIGH-THROUGHPUT GENE ARRAY QPCR

Applied Biosciences gene-chip analysis was carried out on the 3 sites chosen for the susceptibility assays.
Data shows the presence of resistance gene profiles in core depths obtained from each site.

All values represent genes/16S-rRNA (total bacteria) that have been log-transformed (e.g., -1 = 10%, -2 = 1%, -3 = 0.1% population with the gene).

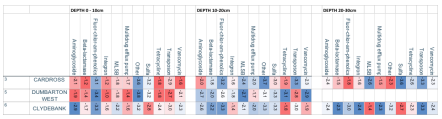


Table 2 - Heat Map of antibiotic resistance genes in 3 chosen sites of River Clyde, UK. Ultra blue = lowest value for the gene cluster among all sites/depth, Ultra red = highest value for the gene cluster.

CONCLUSIONS

- From the results obtained it is clear that gram-negative bacteria isolated from an area with an extensive industrial pollution history show higher minimum inhibitory concentrations and minimum bactericidal concentrations to a range of both PTEs and antibiotics.
- In general, genes for resistance mechanisms were shown to be highest within 0-10 cm of soils however when examining data from deeper cores, isolated bacteria still harbour resistance traits to both PTEs and antibiotics.
- Through a combination of qPCR, high throughput gene array qPCR technology and susceptibility assay data, it is clear co-selection of PTEs and antibiotic resistance does occur, and this impacts bacteria that are potential human pathogens.